PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference Case 21404	**FOR FURTHER ACTION**	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
International application No. PCT/EP 03/10295	International filing date *(day/month/year)* 16.09.2003	Priority date *(day/month/year)* 27.09.2002
International Patent Classification (IPC) or both national classification and IPC C12P7/26		
Applicant DSM IP ASSETS B.V. et al.		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 8 sheets, including this cover sheet.

   ☐ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the opinion
   - II ☐ Priority
   - III ☐ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☐ Lack of unity of invention
   - V ☒ Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
   - VI ☐ Certain documents cited
   - VII ☐ Certain defects in the international application
   - VIII ☐ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
11.03.2004	07.12.2004
Name and mailing address of the international preliminary examining authority: European Patent Office - P.B. 5818 Patentlaan 2 NL-2280 HV Rijswijk - Pays Bas Tel. +31 70 340 - 2040 Tx: 31 651 epo nl Fax: +31 70 340 - 3016	Authorized Officer van de Kamp, M Telephone No. +31 70 340-2373

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/EP 03/10295

I. Basis of the report

1. With regard to the **elements** of the international application *(Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17))*:

Description, Pages

1-9 as originally filed

Claims, Numbers

1-11 as originally filed

Drawings, Sheets

1/1 as originally filed

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

   These elements were available or furnished to this Authority in the following language: , which is:

   ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).

   ☐ the language of publication of the international application (under Rule 48.3(b)).

   ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

   ☒ contained in the international application in written form.

   ☒ filed together with the international application in computer readable form.

   ☐ furnished subsequently to this Authority in written form.

   ☐ furnished subsequently to this Authority in computer readable form.

   ☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

   ☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

   ☐ the description, pages:

   ☐ the claims, Nos.:

   ☐ the drawings, sheets:

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)).

   (Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)

6. Additional observations, if necessary:

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	1-6,9-11
	No:	Claims	7,8
Inventive step (IS)	Yes:	Claims	3-6,10,11
	No:	Claims	1,2,7,8,9
Industrial applicability (IA)	Yes:	Claims	1-11
	No:	Claims	

2. Citations and explanations

   see separate sheet

V. Reasoned statement (Continuation)

2.1 CITATIONS

Reference is made to the following documents:

D1: WADA M ET AL: 'Purification and characterization of monovalent cation-activated levodione reductase from Corynebacterium aquaticum M-13', APPLIED AND ENVIRONMENTAL MICROBIOLOGY, vol. 65, no. 10, October 1999, pages 4399-4403

D2: EP-A-1 122 315 (HOFFMANN LA ROCHE) 8 August 2001

D3: EP-A-1 074 630 (HOFFMANN LA ROCHE) 7 February 2001

D4: EP-A-1 026 235 (HOFFMANN LA ROCHE) 9 August 2000

D5: WANNER P ET AL: 'Purification and characterization of two enone reductases from *Saccharomyces cerevisiae*', EUROPEAN JOURNAL OF BIOCHEMISTRY, vol. 255, no. 1, July 1998, pages 271-278

D2, D3 and **D4** have been cited by the applicant in the application.

2.2 NOVELTY (Art. 33(2) PCT)

2.2.1 *Claims 7 and 8*

D1 discloses purified levodione reductase from *Corynebacterium aquaticum*, reducing levodione to actinol. Moreover, the disclosed levodione reductase is also able to reduce ketoisophorone (Table 3), the enzyme thus also being entitled to the name ketoisophorone reductase. Thus a method of determining the substrate specificity of the purified levodione reductase using ketoisophorone as substrate (Table 3) falls within the terms of **claim 7** as subsequent steps of reducing ketoisophorone and levodione take place simultaneously. (Note that the use of optional terms such as 'e.g.' and 'such as' renders the subject-matter following them non-limiting.) The conditions of the enzyme assay (**D1**, page 4399 right-hand column lines 19-31) fall within the conditions claimed in dependent **claim 8**.

2.2.2 The present application therefore does not satisfy the criterion set forth in Article 33(2) PCT as the subject-matter of **claims 7 and 8** can not be considered as new in respect of the prior art as defined in the regulations (Rule 64(1)-(3) PCT).

2.2.3 *Claims 1-6, and 9-11*

The present application satisfies the criterion set forth in Article 33(2) PCT insofar as the subject-matter of **claims 1-6, and 9-11** can be considered as new in respect of the prior art as defined in the regulations (Rule 64(1)-(3) PCT).

2.3 INVENTIVE STEP (Art. 33(3) PCT)

2.3.1 *Claims 1, 2 and 9*

Document **D2** is considered to represent the closest prior art with respect to the subject-matter of **claims 1, 2 and 9**. It discloses a process for producing actinol from levodione comprising contacting levodione with a recombinant microorganism (*Escherichia coli*) transformed with a levodione reductase-encoding gene from *Corynebacterium aquaticum* AKU611 (cf. example 5(2)). The subject-matter of **claims 1 and 9** differs in that the recombinant host microorganism is capable of reducing ketoisophorone to levodione, with as technical effect that a one-step process is provided for the direct production of actinol from ketoisophorone.

2.3.2 The remaining technical problem to be solved by the subject matter of **claims 1 and 9** may therefore be regarded as the provision of a process for producing actinol, involving a step of reducing ketoisophorone to levodione. The solution would be the use of a host microorganism which is capable of reducing ketoisophorone to levodione.

2.3.3 This solution cannot however be considered as involving an inventive step (Article 33(3) PCT) for the following reasons:

D3 discloses a process for producing levodione from ketoisophorone, comprising contacting ketoisophorone with a microorganism selected from the group of species consisting of microorganisms of the genera *Saccharomyces*, *Zygosaccharomyces* and *Candida*, such as Baker's yeast, *S. cerevisiae* ATCC 7754 *S.* (or *Z.*) *rouxii* HUT 7191, *S. delbrueckii* HUT 7116, *S. delbrueckii* HUT 7102, *S. willianus* HUT 7106, *Z. bailii* ATCC11486 and *C. tropicalis* IFO 1403 (cf. Table 1).

The skilled person, in order to solve the problem of providing a process for

producing actinol involving a step of reducing ketoisophorone to levodione, would seriously contemplate to use a strain as disclosed in **D3** as a host microorganism to transform it with a levodione reductase-encoding gene, substituting it for *E. coli* as a host microorganism in a process for producing actinol from levodione as disclosed in **D2** example 5(2)), thus arriving at the solution as claimed in **claims 1 and 9** of the current application without applying inventive skill and with a reasonable expectation of success.

2.3.4 Dependent **claim 2** does not appear to contain any additional features which, in combination with the features of any claim to which it refers, involve an inventive step, for the reason that the reaction conditions as disclosed in **D2** (e.g., example 5(2)) and **D3** (e.g., claim 7) fall within or overlap with the ranges of claim 2.

2.3.5 *Claims 7 and 8*

Document **D4** is considered to represent the closest prior art with respect to the inventivity of the subject-matter of **claims 7 and 8**. It discloses a process for producing actinol from levodione comprising contacting levodione with a purified levodione reductase isolated from *C. aquaticum* AKU611. The subject-matter of **claim 7** differs in that in addition to purified levodione reductase, purified ketoisophorone reductase is present, with as technical effect that a one-step process is provided for the direct production of actinol from ketoisophorone

2.3.6 The remaining technical problem to be solved by the subject matter of **claim 7** may therefore be regarded as the provision of a process for producing actinol, involving a step of reducing ketoisophorone to levodione. The solution would be the use of purified ketoisophorone reductase alongside purified levodione reductase in a process for producing actinol.

2.3.7 This solution cannot however be considered as involving an inventive step (Article 33(3) PCT) for the following reasons:

D5 discloses two enone reductases from *S. cerevisiae* capable of reducing ketoisophorone (compound 9 in Table 3) yielding levodione (cf. page 277 left-hand column line 64 - right-hand column line 3).

The skilled person, in order to solve the problem of providing a process for

producing actinol involving a step of reducing ketoisophorone to levodione, would seriously contemplate to use a combination of purified levodione reductase as disclosed in **D4** with purified ketoisophorone reductase as disclosed in **D5**, thus arriving at the solution as claimed in **claim 7** of the current application without applying inventive skill and with a reasonable expectation of success.

2.3.8 Dependent **claim 8** does not appear to contain any additional features which, in combination with the features of any claim to which it refers, involve an inventive step, for the reason that the reaction conditions as disclosed in **D4** and **D5** fall within or overlap with the ranges of claim 8.

2.3.9 The present application does therefore not satisfy the criterion set forth in Article 33(3) PCT and the subject-matter of **claims 1, 2, 7, 8 and 9** does not involve an inventive step (Rule 65(1)(2) PCT).

2.3.10 *Claims 3-6, 10 and 11*

Insofar as the subject-matter of **claims 3-6, 10 and 11** is concerned, the present application does satisfy the requirements of Article 33(3) PCT. The subject-matter of these claims involves an inventive step, because it would not be obvious, in view of the prior art, to try to solve the problem of providing a process for producing actinol, involving a step of reducing ketoisophorone to levodione, or a related problem, by using a recombinant microorganism capable of reducing levodione to actinol and transforming it with a ketoisophorone reductase-encoding gene (**claims 3, 4 and 10**), or by using a recombinant microorganism expressing both ketoisophorone reductase- and levodione reductase-encoding genes (**claims 5, 6 and 11**), with a reasonable expectation of success.

2.3.11 **Note, however, that the subject-matter of claims 3-6, 10 and 11 with respect to a ketoisophorone reductase-encoding gene, in conjunction with the description page 5 lines 4-13, appear to contravene Articles 5 and 6 PCT**, in particular the requirements that the invention shall be disclosed by the description in a manner sufficiently clear and complete to be carried out by a person skilled in the art, and that the claims shall be fully supported by the description. Since a ketoisophorone reductase-encoding gene is neither known, nor is it obvious for the skilled person how to obtain such a gene, the subject-matter of **claims 3-6, 10 and 11** appears to reflect

a mere desideratum, suffering from lack of disclosure in the description and lack of support for the claims.

2.4 INDUSTRIAL APPLICABILITY (Art. 33(4) PCT)

2.4.1 The subject-matter of **claims 1-11** satisfies the criterion set forth in Art. 33(4) PCT in conjunction with Rule 5(vi) PCT with respect to industrial applicability.